

WHAT IS CLAIMED IS:

1. An antibody variant of a parent antibody, which antibody variant comprises an amino acid insertion in or adjacent to a
5 hypervariable region of the parent antibody and has a binding affinity for a target antigen which is at least about two fold stronger than the binding affinity of the parent antibody for said antigen.
- 10 2. The antibody variant of claim 1 which has an amino acid insertion in a hypervariable region of the parent antibody.
- 15 3. The antibody variant of claim 1 wherein the hypervariable region is Complementarity Determining Region (CDR) H3 of a heavy chain variable domain of the parent antibody.
- 20 4. The antibody variant of claim 1 wherein about one to about 30 amino acid residues have been inserted in or adjacent to the hypervariable region of the parent antibody.
5. The antibody variant of claim 4 wherein about two to about ten amino acid residues have been inserted in or adjacent to the hypervariable region of the parent antibody.
- 25 6. The antibody variant of claim 1 which has a binding affinity for said antigen that is at least about five fold stronger than the binding affinity of the parent antibody for said antigen.
- 30 7. The antibody variant of claim 1 wherein the antibody variant has a potency in a biological activity assay which is at least about 20 fold greater than the potency of the parent antibody in the biological activity assay.
- 35 8. The antibody variant of claim 7 wherein the potency of the antibody variant in the biological activity assay is at least about

50 fold greater than the potency of the parent antibody in the biological activity assay.

9. The antibody variant of claim 1 wherein the parent antibody is
5 a humanized antibody.

10. The antibody variant of claim 1 wherein the parent antibody is
a human antibody.

11. The antibody variant of claim 1 wherein at least one of the
10 inserted residues has a net positive charge or a net negative
charge.

12. The antibody variant of claim 11 wherein at least one of the
15 inserted residues is arginine or lysine.

13. The antibody variant of claim 3 wherein the insertion is
adjacent to residue number 100 of the heavy chain variable domain
of the parent antibody, utilizing the variable domain residue
20 numbering as in Kabat.

14. The antibody variant of claim 13 wherein the insertion
consists of about three inserted amino acid residues.

25 15. The antibody variant of claim 1 further comprising an amino
acid substitution in the hypervariable region.

16. The antibody variant of claim 1 which comprises a heavy chain
variable domain, wherein CDR H3 of a heavy chain variable domain of
30 the variant antibody comprises the amino acid sequence of SEQ ID
NO:85.

17. The antibody variant of claim 16 which comprises a heavy chain
variable domain comprising the amino acid sequence in SEQ ID NO:98
35 or SEQ ID NO:99.

18. A composition comprising the antibody variant of claim 1 and a pharmaceutically acceptable carrier.

5 19. An antibody variant comprising a heavy chain variable domain, wherein CDR H3 of the heavy chain variable domain comprises the amino acid sequence of CDR H3 of a variant selected from the group consisting of Y0239-19 (SEQ ID NO:85); Y0239-8 (SEQ ID NO:53); Y0240-1 (SEQ ID NO:86); Y0239-12 (SEQ ID NO:78); Y0239-9 (SEQ ID
10 NO:54); and Y0261-6 (SEQ ID NO:89).

20. A method for producing an antibody variant comprising introducing an amino acid residue in or adjacent to a hypervariable region of a parent antibody, wherein the antibody variant has a
15 binding affinity for a target antigen which is at least about two fold stronger than the binding affinity of the parent antibody for said antigen.

21. The method of claim 20 wherein the hypervariable region in
20 which the amino acid residue is introduced is one which is involved in binding the antigen in the parent antibody.

22. A method for making an antibody variant, comprising the steps of:

25 (a) identifying potential amino acid interactions between a hypervariable region of a parent antibody and a target antigen;
 (b) preparing a variant of the parent antibody comprising introducing an amino acid residue in or adjacent to the hypervariable region of the parent antibody, wherein the introduced
30 amino acid residue contributes to the potential amino acid interactions in (a); and
 (c) selecting an antibody variant prepared as in (b) which has a stronger binding affinity for said antigen than the parent antibody.

23. The method of claim 22, wherein step (a) involves analyzing a molecular model of the parent antibody complexed with said antigen.

24. The method of claim 22 wherein step (b) comprises preparing
5 antibody variants displayed on phage.

25. The method of claim 22 wherein the amino acid interactions are selected from the group consisting of hydrogen-bonding, Van der Waals interactions and ionic interactions.

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26. Isolated nucleic acid encoding the antibody variant of claim
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27. A vector comprising the nucleic acid of claim 26.

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28. A host cell transformed with the vector of claim 27.

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29. A process of producing an antibody variant comprising culturing the host cell of claim 28 so that the nucleic acid is expressed.

30. The process of claim 29 further comprising recovering the antibody variant from the host cell culture.

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31. The process of claim 30 wherein the antibody variant is recovered from the host cell culture medium.